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# A NOVEL STRATEGY FOR INVESTIGATING THE MECHANISM OF DNA INTERSTRAND CROSS-LINK REPAIR IN MAMMALIAN CELLS.

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DNA cross-linking agents are clinically important chemicals. Their application towards the treatment of both solid tumours and leukaemias as well as psoriasis has been extensive. Increasingly, the role of cross-link repair in tumor cell resistance to these compounds has become evident. Moreover, the high efficiency of cross-link repair mechanisms in normal cells is illustrated by the exquisite sensitivity of chinese hamster cell mutants that are complemented by several genes (*ERCC1*, *ERCC4/XPF*, *XRCC2*, and *XRCC3*). In spite of recent progress outlining the mechanism of nucleotide excision repair, the steps involved in DNA interstrand cross-link repair remain largely unknown. Homologous recombination between sister duplexes must likely be involved for the process to occur in an error-free manner. A novel strategy is being devised to investigate the molecular mechanism of DNA interstrand cross-link repair in mammalian cells and the role of homologous recombination.

We plan to introduce into cells a matched pair of plasmids that carry cross-links in either the 5' or 3' end of a target reporter gene (hygromycin [*hyg*] resistance), with sufficient intervening sequence to allow homologous recombination to occur between the regions of damage. Detailed sequence analysis of the 1026 bp *hyg* gene identified restriction enzyme sites that allow us to remove small portions (~80 bp) of both the 5' or 3' ends in regions that overlap the start and stop codons. We will introduce cross-links into these defined regions by cross-linking synthetic oligonucleotide duplexes and ligating them into the vector. The *hyg* gene was amplified by PCR and inserted into the multi-cloning site of the mammalian expression vector pcDNA3, forming the construct pcDNA3H that was verifed as having functional *neo* and *hyg* genes. We are determining the conditions for cross-linking the oligonucleotide duplexes using 4, 5, 8-trimethylpsoralen (TMP). The DNA is mixed with TMP (10 μg/ml) and irradiated at 366 nm using a UV laser at a power of 20 mW/cm$^2$ for up to 60 min. The products are purified, end-labelled using the T4 polynucleotide kinase exchange reaction, and analysed by electrophoresis to determine the optimal conditions for interstrand cross-link formation. The next step is to remove a restriction fragment from the 5' end of the *hyg* gene in pcDNA3H and replace it with a cross-linked fragment. This ligation product will be transfected into cells, and procedures will be devised to identify molecules that have undergone the unhooking (initial) step in cross-link repair. In subsequent studies involving co-transfection with the two damaged plasmids that can recombine we will attempt to demonstrate recombinational repair. This work was carried out under the auspices of the US DOE by LLNL under contract No. W-7405-ENG-48.